FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

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Potential roles of cell-derived microparticles in Multiple Sclerosis

Potenciais implicações das micropartículas celulares na Esclerose Múltipla

março, 2018

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Mestrado Integrado em Medicina

Área: Neurologia

Tipologia: monografia

Trabalho efetuado sob a Orientação de:

Professora Doutora Joana Cruz Guimarães Ferreira Almeida

Trabalho organizado de acordo com as normas da revista:

International Journal of Clinical Neurosciences and Mental Health

março, 2018

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Neurologia

TÍTULO MONOGRAFIA

Potential roles of cell-derived microparticles in multiple sclerosis.

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Dedico este projeto aos meus pais e irmão, pela capacidade de acreditar e investir em mim sem medir esforços para que eu chegasse até esta etapa da minha vida.

Em especial, gostaria de agradecer à minha mãe, por ser uma referência na minha vida e pelo cuidado e dedicação que tornaram este percurso melhor.

Aos meus amigos, por serem um pilar fundamental na minha vida e me acompanharem neste caminho tornando-o mais leve.

Potential roles of cell-derived microparticles in Multiple Sclerosis

Abstract

Multiple Sclerosis (MS) is a chronic, inflammatory and immune-mediated disease of the Central Nervous System (CNS). The aetiology of MS remains unclear but probably represents the interaction between genetic factors, environmental triggers, and an imbalanced immune system. The term microparticles (also known as microvesicles) refers to a heterogeneous population of vesicles with a diameter ranging from 0,1 to 1 µm that are released by budding directly from the cell membrane along with subsequent fission on the membrane stalks and express antigens specific of their parental cells. Despite being a physiological process, generation of microparticles is associated with multiple pathologic conditions, including inflammatory and autoimmune diseases, atherosclerosis and malignancies. Understanding the aspects involved in the dynamics of microvesicles could then provide new perspectives into MS disease mechanisms and give rise to new diagnostic tools and therapeutic targets. The present review summarizes current knowledge about the impact of microparticles in MS pathophysiology and its diverse potential roles, as well as exploring eventual improvements in medical treatment.

Introduction

Microparticles (also known as microvesicles) are small bilayer vesicles ranging from 0,1 to 1 µm in diameter which corresponds approximately to 1/7 the diameter of a human erythrocyte [1]. Along with exosomes and apoptotic blebs, microparticles are classified as extracellular membrane vesicles (EMV) [2]. Upon certain conditions such as cell stimulation, stress or activation these vesicles are shaped by budding directly from the cell membrane along with subsequent fission on the membrane stalks [3]. Among mammals, microparticles (MPs) are produced by almost all types of cells, including circulating blood cells (platelets, erythrocytes, neutrophils, lymphocytes and monocytes/macrophages), vascular cells (endothelial cells, smooth muscle cells and

fibroblasts) and cells belonging to other tissues or organs in physiological and pathological conditions (including autoimmune diseases) [4].

The importance of MPs has once been neglected taking into account that they were considered as cell “dust” or “debris” resulting from cellular processes [5]. However, according to the latest findings one may suspect important roles in various pathological states namely autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous, Sjögren’s Syndrome [6] and, regarding the Central Nervous System (CNS), Multiple Sclerosis [7]. It is known that MPs carry cytokines and other molecules with biological activity, such as immunocomplexes, but also proteins and nucleic acids, so enhancing the transmission of cellular information including autoimmune responses. Microvesicles have negatively charged membranes and carry membrane markers from the parent cell which means it is possible to identify its specific cellular origin [8].

Circulating MPs may be fundamental intervenients in intercellular communication during inflammation [9]. In fact, they take part in both local and distant cell-to-cell signalling and promote the exchange of cytoplasmic proteins and large surface molecules (such as receptors and complement proteins) between cells and therefore enhancing both direct cell-to-cell and cytokine-mediated communication processes. Notably, these small vesicles have a greater lifespan and spread more widely than their parent cells [4]. Understanding the aspects involved in the dynamics of microvesicles could then provide new perspectives into disease mechanisms and give rise to new diagnostic tools and therapeutic targets considering the possibility of using them as new biomarkers for monitoring the disease under study.

Multiple Sclerosis (MS) is a chronic, inflammatory and immune-mediated disease of the CNS. The aetiology of MS remains unclear but there is evidence it involves the interaction between genetic factors, environmental triggers and an imbalanced immune system [10]. It affects more than 2 million people worldwide, with women being more

susceptible (approximately 3:1) [11]. Disease course and symptoms are highly heterogeneous and it is characterized by the presence of inflammation, demyelination and axonal damage upon the brain and the spinal cord. Disease onset usually occurs in young adults being that the majority of patients (80%) present with a relapsing and remitting course (RRMS) with the rest having a primary progressive clinical course (PPMS). The secondary progressive form (SPMS) occurs as a second phase of the disease for those who initially had RRMS and is characterized by a progressive worsening of neurologic function [12]. Given its high prevalence, MS is the leading cause of neurological damage from non-traumatic causes in young adults in the United States and Europe [13] and thus justifying the importance of its study.

Despite clinical studies concerning MP analyses now have greater numbers than they used to, comparatively few have specifically addressed neurological conditions. The present report is therefore, a possible tool for better understanding the nature of MPs involvement in neurological conditions, namely in Multiple Sclerosis, and to explore the role of MPs as prognostic markers, biological agents and also potential therapeutic targets.

Cellular microparticles

Differently from exosome shaping, in which inward membrane invagination occurs, MP formation requires outward blebbing of the plasma membrane and the latter are also known as “ectosomes” [14]. Notably, the precise mechanisms leading to vesiculation remain unclear. However, there are two essential steps during this process: cytoskeletal reorganization and alterations in phospholipid symmetry. Cell membrane in its resting state is characterized by the distribution of its phospholipids, with phosphatidylcholine and sphingomyelin situated on the outer side, and phosphatidylethanolamine on its internal layer [15]. This asymmetry is guaranteed by an active transmembrane enzymatic balance related to an equilibrium between flippase, floppase and scramblase [16].

When a cell undergoes activation or apoptosis processes there is a sudden release of intracellular calcium by the endoplasmic reticulum. This immediate rise in cytosolic calcium leads to the translocation of phosphatidylserine (PS) from the inner leaflet to the cell surface and activation of cytosolic enzymes such as calpain resulting in the cleavage of cytoskeleton filaments [17]. As a consequence, membrane-derived MPs bleb and shed into the extracellular fluid. It is interesting to note that MP membranes express not only phosphatidylserine but also various phospholipids and oxidized lipids along with a wide range of proteins specific for cell type from which they originate making heterogeneity a hallmark of microparticles [18]. In addition, the stimulus responsible for the induction of vesiculation determines the lipid and protein composition of MPs. Considering the same cell type receiving different stimuli will result in microparticles that have distinct components. Furthermore, different cell types exposed to the same stimulus will produce different microparticles. In fact, there are plenty of stimuli which might stimulate or inhibit MP formation. Chemical and even physical stimuli can trigger microparticle liberation, namely cytokines, immunocomplexes, ultraviolet-B (UVB) light, tobacco smoke extract, unesterified cholesterol, thrombin, endotoxins and shear stress [4]. Interestingly, it has been reported that statin treatment and Nitric Oxide (NO) suppress MP production from endothelial cells. To sum up, all are potentially capable of taking part in the development of autoimmune diseases or its complications.

In contrast with their formation, much less is known about the mechanisms of MP elimination. Regarding this process, further investigation is required in order to better comprehend the mechanisms behind the removal of various membrane vesicle populations. In regular adults, over 10^9 cells die each day and each apoptotic cell liberates several microparticles [19]. As a matter of fact, a correlation between autoimmune diseases and defects in the clearance of apoptotic cells and their microvesicles has been found [20],[21].

Phagocytosis is strongly believed to be the pivotal mechanism responsible for elimination of MPs with these membrane vesicles being engulfed within phagocytic cells [22]. Under normal conditions, PS is confined to the internal leaflet of the plasma membrane but as long as there are apoptotic stimuli to a specific cell, it changes to a superficial position and is then recognized by phagocytes [23] that apparently express several molecules responsible for that process, for instance lactadherin, developmental endothelial locus-1 (DEL-1), alpha (v) integrin/MFG-E8 (milk fat globule epidermal growth factor), Mer/Gas6, stabilin-2, brain angiogenesis inhibitor 1 (BAI-1) and T-cell immunoglobulin and mucin domain 4 (TIM-4) that bind PS either directly or through bridging molecules [24], [25]. Externalization of PS seems to be responsible for the consequent phagocytosis and internalization of MPs by signalling scavenger receptors which might induce endocytosis/efferocytosis of MPs.

Aside from PS, opsonisation by IgM may also work as a facilitator mechanism for the binding and uptake of MPs by macrophages [26]. It is worth to note that glycosylation patterns might actually work as a means of distinction and prioritization for the clearance of MPs and other extracellular vesicles by macrophages. On the other hand, there is a pattern-recognition molecule, complement C1q which has also been related to microvesicle clearance [27]. Circulating plasma MPs concentration is a result from the balance between its production and clearance.

Methods

In order to gather information about MPs and its impact in MS, during the initial phase of this project and before writing the current report, the first step was to perform a literature review by selecting studies using the electronic database MEDLINE/PubMed according to specific key words previously determined: “Multiple Sclerosis”, “Multiple Sclerosis” and “Microparticles” OR “Microparticles” and “Biomarker”. When scanning the tittle and abstract of studies, some were promptly considered irrelevant for this review and therefore excluded. After analysing the reference list from articles found

with the computerized research additional studies have also been selected. All the papers included were published in English. Finally, the reading of the identified relevant articles was conducted.

MPs as active mediators in MS progression

Tight junctions between cerebrovascular endothelial cells are key factors in maintaining homeostasis and ensuring the integrity of blood-brain barrier (BBB) [28]. Under the unique environment of the CNS, a paracellular diffusion barrier at the vascular interface with circulating blood is assembled by these highly specialized complexes, which are partially regulated by astrocytic foot processes, pericytes, neurons and also extracellular matrix. Inflammation is strongly correlated with mechanisms of tight junction deregulation and contribution to loss of barrier function in CNS.

MS is considered to be a chronic inflammatory disease of the CNS, which causes a heterogeneous range of signs and symptoms due to the fact that a differential involvement of motor, sensory, visual and autonomic systems is present. It is of note that amidst the initial and most solid abnormalities of the brain of MS patients are changes in the BBB and migration of inflammatory cells across the endothelial barrier [18]. Disrupting the barrier might be a fundamental step in the emergence of demyelinating lesions. Bearing this in mind, one might say that endothelial barrier dysfunction is an imprint of MS. This infirmity has a characteristic relapsing clinical course and its pathology is intimately linked with the perivenular leukocyte infiltration and demyelination in the CNS. In fact, MP formation in plasma is a forehand and lasting outcome of demyelinating events.

The involvement of MPs in several pathological processes appears to be more complex than initially thought and concerning to MS it has been reported that plasma MPs induce human endothelial barrier dysfunction and therefore might have an active function in its progression [2]. The origin of MPs increase is likely related with the permanent exposure to various inflammatory mediators that markedly display a

significantly long-term function in the advance of disease. Additionally, these mediators may also amplify MPs effect on endothelium and since endothelium is strongly exposed to MPs, its cells have greater odds of reacting to this MP-mediated signalling. Signals that damage BBB function are formerly originated from CNS. During initial stages of MS there is a release of reactive oxygen species (ROS), tumour necrosis factor (TNF) and interferon gamma (IFN-y) by microglia, which can all induce MPs formation and liberation. What is more, microvesicles (MVs) from the brain endothelium have the capability of activating both CD4+ and CD8+ T cells toward neuronal antigens when lacking any other stimulatory signal and might portray the inaugural step of brain autoimmunity [29].

To address the relevance of MPs it is necessary to explain the different mechanisms by which they influence the endothelial barrier function. One of the forms of regulation of endothelial permeability is correlated with certain surface receptors expressed by MPs [30]. A commonly used marker for the identification of MPs from endothelial origin (EMPs), known as platelet and endothelial cell adhesion molecule (PECAM)-1/CD31, is capable of establishing homotypic interactions and hence modulating endothelial permeability. On the other hand, EMPs also take part in the regulation of the production of ROS which induce disruption of the endothelial barrier when augmented. Another aspect of the repertoire of the influence of MPs in the endothelial barrier consists in the local release of cytokines and consequent increase in its permeability, thus acting as pro-inflammatory agents. As an example, it is worth to note that MPs from microvascular endothelial origin and also from atherosclerotic plaques actually contain matrix metalloproteinases (MMPs) implicated in the cleavage and shedding of surface proteins, for instance TNF [30]. As for the ones that derive from monocytes, they contain inflammatory cytokines that in like manner have the potential to modulate permeability. As a matter of fact, these same MPs induce the activation of the transcription factor NF-kB and the expression of adhesion receptors. Finally, MPs also

constitute vehicles of transportation of RNA and micro(mi)RNA, which can potentially modulate protein expression in the target cell [17]. It has been demonstrated that some miRNAs, for example miRNA155, has a negative impact in BBB function during episodes of neuroinflammation [30]. Taking these facts into consideration, further studies addressing the analysis and comparison of the distinct components between healthy and MS microparticles are necessary and could give rise to new targets for preventing endothelial barrier disruption that occurs concomitantly with the progression of the disease.

MPs might induce changes in vasculature either by themselves, at higher concentrations, or by potentiating the outcomes of proinflammatory mediators, such as Thrombin, when acting synergically at lower concentrations, important for MS onset. The role of Thrombin in the inflammatory development of experimental MS has been proven [31]. Indeed, Thrombin is an inflammatory mediator that promotes barrier contraction and in the long term causes activation of the endothelium due to inflammation. According to these findings, one may suspect that MPs with no clear effect on endothelial barriers on their own, might act as sensitizing agents acting on cells and consequently amplifying the barrier disruption mediated by Thrombin. There is a link between MS pathophysiology and coagulation that justifies the greater abundance of certain proteins involved in this cascade, including tissue factor (TF), responsible for the activation of thrombin, in chronic active plaques. Thrombin is a cytokine with an unquestionable ability to signal and modify the barrier properties of endothelial cells, occupying a crossing position between inflammation and coagulation and thereby perhaps contributing to the progression of several pathologies, including MS, as revealed by the fact that platelet-MPs (PMPs) might activate leukocytes and promote their transendothelial migration. Furthermore, MPs have phosphatidylserine as a component of the outer part of the membrane and so they actually promote a procoagulant status.

As stated previously, in MS patients, MPs alone are capable of increasing extravasation of molecules and cells from the bloodstream in a long term perspective [30]. Another point is that this effect and even the composition of these vesicles are different between MS patients and healthy individuals.

MPs as Biomarkers

First of all, it becomes necessary to better perceive the concept of a biomarker as they correspond to measurable and quantifiable biological features that can then be appraised as indicators of regular biological processes, pathological processes or pharmacological responses to medical treatment [16]. Using biomarkers it is possible to identify pathological conditions and assess the risk of disease, thus definitely being instruments of great clinical utility.

The hypothesis of resorting to MPs as markers of various pathological processes, including MS, has been already posed although the potential function of these vesicles is not fully characterized [32]. A pertinent question is whether MPs are correlated with the progressive forms of the disease or with any form in particular. Despite being a recent topic, some studies have been conducted in the sense of seeking to answer these doubts.

Some MPs may move through a diffusion process due to their small size and thus make possible their detection in various biological fluids such as blood, urine and even synovial fluid [17]. Given this possibility, MPs have been highly anticipated as potential markers of disease states capable of providing clinically relevant information. The method used for detection consists of a technique called flow cytometry that allows direct quantification of MPs from biological fluids. Compared to exosomes (50-100nm in diameter), which are below the detection limit of this approach, MPs are of considerably larger size (200-1,000 nm) thus justifying the utility of this method in its quantification. Microglia are immune cells that reside in the CNS and act as agents of first line defence in cerebral pathologies. Interestingly, every report that studied the

possible diagnostic value of MPs in CNS diseases has focused not on microglia but rather on MPs derived from platelets, endothelial cells or oligodendrocytes [33].

A correlation between MPs and neurological episodes from patients diagnosed with the RRMS form appears to exist since, during the relapse phase of these patients, a rise in circulating MPs derived from the endothelial cells has been documented [34]. Not only these patients show an increase in MPs but in fact all the different forms of MS, including the progressive ones, are associated with an increase in the amount of circulating MPs whose origin comes from platelets and endothelial cells [35]. Taking this assumption into consideration, the variation on levels of circulating MPs leads to the conclusion that MS, in all its forms, is associated with platelet and endothelial dysfunction. Interestingly, even patients with typical clinically isolated syndrome (CIS) and already recovered or in the remission phase show a significant elevation in MPs when comparing with healthy subjects [30]. Particularly, the fact that the same finding was also found in CIS patients suggests endothelial dysfunction is present from the very beginning of proinflammatory demyelinating pathologies which means these circulating vesicles remain chronically elevated after developing the first neurological episode. In the same fashion, the vesicles derived from platelets were also found to be significantly elevated in all MS subtypes which corroborates the hypothesis that platelets have an essential role in MS [36] [37]. Nevertheless, in CIS patients the increase in PMPs has not been proven significant and so one might conclude platelet dysfunction occurs posteriorly to the endothelial dysfunction when the disease is indeed moving forward. Interestingly, platelet hyperactivity has been linked to patients with SPMS which even have an increased risk of thromboembolic events due to the greater levels of platelet aggregation and PMP formation [37]. As a result, we might look at EMPs and PMPs as good candidates not only for clinical monitoring of disease state allowing the distinction between CIS and early MS but also for the assessment of progression of disease and response to therapy.

Despite being elevated in all forms of MS and regardless of the phase when considering RRMS, truth be told, the composition of these vesicles is probably different.

During the last decade, several studies have shown that extracellular vesicles (including MPs) harbour many bioactive molecules and exert important regulatory functions on target cells. In fact, there is a profound relationship between the severity of various diseases and the molecular content encapsulated in these vesicles, such as RNA and proteins [38].

Under physiological circumstances the endothelium does not express great levels of immunoglobulin-like adhesion molecules (IgCAMs). Nonetheless, upon stimulation by proinflammatory signals or activated T cells there is an upregulation of IgCAMs on the BBB which might increase penetration of inflammatory cells into the CNS [39]. Particularly in MS, several soluble markers have been reported as being possibly detected in plasma or cerebrospinal fluid (CSF). Some examples important to highlight are PECAM-1 (CD31) and also E-Selectin (CD62E) which are present in MPs from endothelial origin either while apoptosis is occurring or after inflammatory stimuli. As a matter of fact, their plasma concentration might reflect the state of endothelial cells or the presence of endothelial damage. In like manner, another important marker is CD51 (integrin $\alpha_{v\beta3}$) also found in EMPs.

Levels of soluble PECAM-1 are thought to be an index of BBB disruption in MS, as can be seen by the confirmation of higher levels of CD31+ EMPs during exacerbation but not remission phase [40]. Having this in mind, PECAM-1 is apparently connected with clinical relapses along with the presence of gadolinium-enhancing lesions. Brain magnetic resonance imaging (MRI) has long been settled as the most sensitive in vivo technique for detecting disease activity and BBB damage [41]. However, it is still an expensive and time-consuming method and that is why alternative tools are an

appealing possibility when monitoring disease activity. CD31+ EMP have shown potential utility in detecting MS as a complementary method used concomitantly with MRI with gadolinium [42]. This soluble molecule is involved in transendothelial leukocyte migration characteristic of inflammatory phenomena and is also responsible for the stimulation of B1 and B2- type integrins on leukocytes that leads to an increase in their binding to and migration through endothelium. On balance, PECAM-1 seems to meet the necessary conditions to assert itself as a naturally useful marker of MS activity. Regarding EMPs measured by CD51, although less pronounced than with CD31, elevated levels have been detected not only in exacerbation but also during remission phase [40]. Despite not being exclusive to endothelial cells, CD51 is believed to reflect erosion of endothelium with consequent exposure of subendothelial matrix when elevated and differently from CD31, it seems to behave more like a marker of chronic disease in RRMS, seemingly independent of exacerbation of disease. Yet, the one or more soluble factors present in MS plasma that cause the release of EMPs remain unclear and warrant more attention in future investigations.

Therapeutic Perspectives

MPs have proven themselves as a fascinating target for therapeutic intervention in numerous pathologies. Particularly, management of MS could be significantly improved by novel therapies based on MPs modulation. Equally important would be the potential benefits from the improvement of precocious diagnostic tools that could hasten the beginning of MS treatments. Indeed, MP profile may foreshadow BBB disruption and behave as a “surrogate” marker of vascular stress in MS. Currently, MS remains an incurable condition and existing treatments target the modulation of the immune system mostly with the use of interferon beta (IFN-β), glatiramer acetate, natalizumab and fingolimod [34]. In this regard, although there is still much progress to be done, small but persistent steps are being taken towards the investigation of more efficacious treatment options. One hypothesis would be trying to prevent disruption of the

endothelial barrier with the discovery of new targets after clarifying the differences in composition between MPs from MS patients and healthy subjects. Additionally, a critical step in the dissection of the various biological effects of MPs will lay in the development of selective pharmacological agents which modulate either their formation or, concomitantly or not, their signalling. Some MPs can be harmful and consequently spread inflammation. MP-focused therapy is based on the hypothesis that, by a quantitative or qualitative route, there may be a decrease in its pathogenicity and a possible benefit in the course of the disease.

Fingolimod, the first oral treatment for MS, apparently corrects dysregulated endothelial MP levels, according to a study that found quantitative and qualitative changes in circulating MP numbers from MS patients, that way restoring normal values [43]. The pharmacological control of MPs counts can be viewed as a promising challenge and innumerable possibilities have been posed, such as statin treatment, fibrates, GPIIbIIIa antagonists and other anti-platelet treatments. Anything that promotes MPs release can hypothetically be a target for pharmacological control, including oxidative stress, cytokines and neurohormonal stimulation by angiotensin II and cathecolamines for instance. Lastly, we could consider a more embracing approach by trying to modulate membrane remodelling, and subsequent vesiculation and MPs pouring. In order to achieve that effect we could possibly target any specific or occasional PS membrane translocator such as ATP binding cassette subfamily A member 1 (ABCA1) [44]. Nevertheless, it should be emphasized that, despite huge progress during last decades has been made, there is still much to know and more detailed studies are necessary to better understand whether these possible targets for MPs modulation have the intended effect specifically in MS disease.

Conclusions

In the current report we went through the distinct roles of MPs and exploited its many potential applications. After reviewing the current available literature, one might

conclude MPs studies in neurological conditions, including MS, are essential as a research tool for gaining insights into its pathophysiology and also due to their eventual clinical benefits in the detection, monitoring and possibly therapeutic intervention in MS.

Acknowledgements

I wish to thank Professor Joana Guimarães for the guidance of the project by careful reading of the manuscript and providing helpful discussion concerning the topic and giving wise advice.

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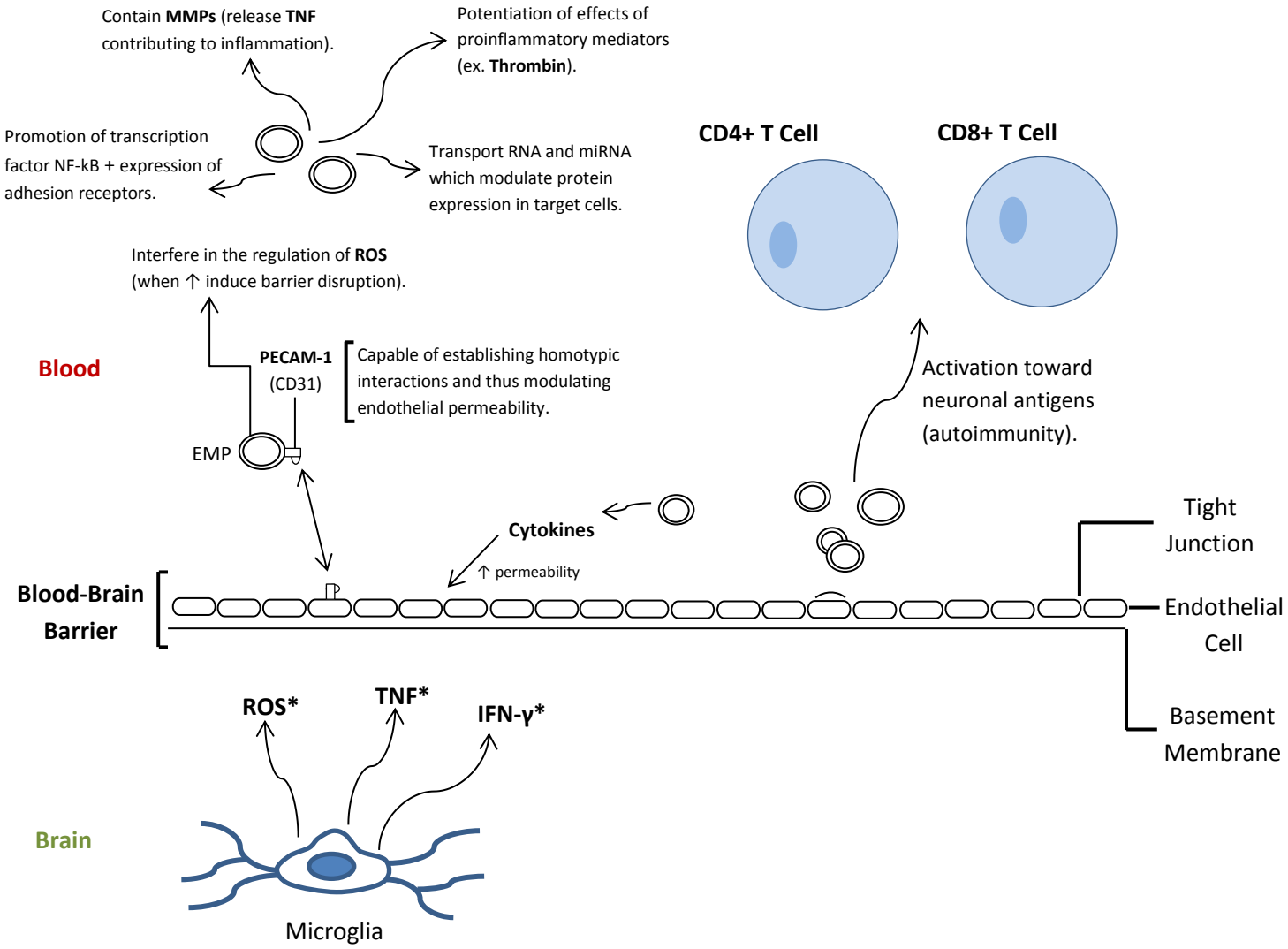

Figure 1 – MPs pathophysiology in Multiple Sclerosis

***** Inflammatory mediators can induce MPs formation and release as well as amplify its effects.
MPs: microparticles
MMPs: matrix metalloproteinases
TNF: tumour necrosis factor
miRNA: microRNA
ROS: reactive oxygen species
IFN-γ: interferon gamma

INSTRUCTIONS FOR AUTHORS

Contents

1. Aims and Scope

The International Journal of Clinical Neurosciences and Mental Health is an open-access peer-reviewed journal published by ARC Publishing.

Our goal is to provide high-quality publications in the areas of Psychiatry and Mental Health, Neurology, Neurosurgery and Medical Psychology. Expert leaders in these medical areas constitute the international editorial board.

The journal publishes original research articles, review articles, drug reviews, case reports, case snippets, viewpoints, letters to the editor, editorials and guest editorials.

The International Journal of Clinical Neurosciences and Mental Health follows the highest scientific standards, such as the CONSORT / STROBE guidelines and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (ICJME).

The journal offers:

- Trusted peer review process
- Fast submission-to-publication time
- Open-access publication without author fees
- Multidisciplinary audience and global exposure

2. Types of papers

The International Journal of Clinical Neurosciences and Mental Health publishes scientific articles in the following categories:

- Original Research.
- Reviews.
- Drug Reviews.
- Case Reports.
- Case Snippets.
- Viewpoints.
- Letters to the Editor.
- Editorials and Guest Editorials.

As an open-access, online-only publication, the International Journal of Clinical Neurosciences and Mental Health does not enforce arbitrary word count or illustration limits. The journal a provides a recommendation on the length of manuscripts, but authors are welcome to submit manuscripts outside those recommendations if deemed appropriate.

2.1. Original Research

The International Journal of Clinical Neurosciences and Mental Health welcomes original clinical or translational research related with psychiatry, mental health, medical psychology, neurosurgery and neurology.

Reports of randomised clinical trials should follow the CONSORT Guidelines and reports of observational studies should follow with STROBE Guidelines.

Original Research articles are recommended to have up to 4000 words (excluding title page, abstract, acknowledgements, references and tables) and up to 8 illustrations (figures or tables). Submission of supplementary material is encouraged. This may include additional illustrations of study results (both figures and/or tables), video files presenting study results or procedures, study protocol, study database and statistical analysis plan.

2.2. Reviews and Drug Reviews

Review articles on current topics related to psychiatry, mental health, medical psychology, neurosurgery and neurology, as well as CNS-related drugs are welcome. Both invited and unsolicited submissions are accepted.

Review articles are recommended to have up to 5000 words (excluding title page, abstract, acknowledgements, references and tables.). Inclusion of newly designed figures and tables to summarise key points is encouraged. The used of previously published material is subject to the licence agreement of the original publisher, and should generally be avoided. If previously published materials are, nonetheless, included in the illustrations, the authors should procure appropriate authorisation for use from the original publisher prior to submission.

2.3. Case Reports and Case Snippets

Highly meaningful Case Reports are accepted, presenting major educational content or major clinical findings. Case Snippets should describe a diagnosis or therapeutic challenge.

Case Reports and Case Snippets are recommended to have 750–1000 words (excluding title page, abstract, acknowledgements, references and tables) and up to 2 figures or tables.

2.4. Viewpoints

Viewpoints should provide an expert opinion on important topics for medical research or practice, with possibility for covering social and policy aspects. This section encourages dialogue and debate on relevant issues with expert views based on evidence.

Viewpoints are recommended to have 1500–3000 words (excluding title page, abstract, acknowledgements, references and tables) and can include figures or tables, as deemed appropriate.

2.5. Letters to the Editor

Letters to the Editor should share views on published articles, any findings insufficient for a research article or present ideas on any subject within the scope of the journal.

Letters to the Editor are recommended to have up to 1500 words (excluding title page, abstract, acknowledgements, references and tables) and can include figures or tables, as deemed appropriate.

2.6. Editorials and Guest Editorials

Authors are invited by the Editor-in-Chief to comment on specific topics and express their opinions in the form of Editorials. Nonetheless, interested authors are encouraged to contact the Editor-in-Chief with proposals for writing Editorials.

3. Manuscript Submission

These instructions advise on how the manuscript should be prepared and submitted. Manuscripts that do not comply with the guidelines will be returned to the authors before being considered for peer-review.

All manuscripts should be prepared in A4-size or US-letter size, in UK or US English throughout the manuscript, a mixture of UK and US English will not be accepted.

Manuscripts should be submitted in *.doc and *.pdf formats, in the appropriate section of the journal website: IJCNMH online submission.

3.1. Cover Letter

A cover letter should be submitted together with the manuscript, in *.doc or *.pdf format, addressed to the Editor-in-Chief, and signed by the author submitting the manuscript.

A template for the cover letter is available for download.

The cover letter should contain statements about originality of your publication, Ethics Committee approval and informed consent (if applicable), conflicts of interest and why in your opinion your manuscript should be published.

3.2. Manuscript Preparation

The manuscript must be divided in 2 files: the Title page (submitted in *.doc format and *.pdf formats) and the Manuscript body (submitted in *.doc and *.pdf formats).

Submitting these 2 files is essential to ensure double-blind peer-review. Failure to provide these 2 files will result in delay in the peer-review process, since the manuscript will be returned to the authors for adjustment.

Title page

This should be submitted as a separate file from your manuscript (to ensure anonymity in the peer review process) and should include:

- Article title.
- Authors' names, titles (e.g. MD, PhD, MSc, etc.) and institutional affiliations.
- Corresponding author: name, mailing address, telephone and fax numbers, email address.
- Keywords (maximum of 10), according to MeSH terms, whenever possible.
- A short title (running head) (up to 70 characters).
- Abstract word count (up to 250 words).
- Disclosure of conflicts of interest. Any conflict of interests should be declared. If authors have no declaration it should be written: "The authors declare no conflict of interest".

Manuscript body:

The Manuscript body must be anonymous, not containing the names or affiliations of the authors. It must be structured in the following order: title, abstract, body text, acknowledgements, references, tables, and figures captions/legends. The manuscript body should contain the title and the abstract, since the title page is not sent to reviewers during peer-review.

- The text must be formatted as follow:
- Arial fonts, size: 11 points.
- Double line spacing (see paragraph menu).
- Aligned to the left (not justified).

Showing continuous line numbers on the left border of the page. For MS Word you can add line numbers by going to: Page Layout -> Line Numbers -> select "Continuous"; for OpenOffice: Tools -> Line Numbering -> tick "Show numbering".

Title

A descriptive and scientifically accurate article title should be provided.

Abstract (250 words maximum)

An abstract should be prepared for all types of manuscript, except Editorials.

Abstracts of Original Research articles should be structured as: background/objective, methods, results, and conclusions. If the publication is associated with a registered clinical trial, the trial registration number should be referred at the end of the abstract.

Case-reports should be structured as background/introduction, case report, discussion.

Systematic review articles should have a structured abstract with generally the same headings as Original Research articles, whereas narrative review articles can have a structured or unstructured abstract, as deemed appropriate by the authors.

Abstracts for Viewpoint articles and Letters to the Editor, can have a structured or unstructured abstracts, as deemed appropriate by the authors.

Body text

Original research articles

Original research articles should be structured as follows:

Introduction: Should present the background for the investigation and justify its relevancy. Claims should be supported by appropriate references. Introduction should end by stating the objectives of the study.

Methods: Should allow the reproduction of results and therefore must provide enough detail. Appropriate subheadings can be included, if needed.

Results: Should include detailed descriptions of generated data. This section can be separated into subsections with concise self-explanatory subheadings.

Discussion: Should be brief but comprehensive and well argued, summarise and discuss

the main findings, their clinical relevance, the strengths and limitations of the study, future perspectives with suggestion of experiments to be addressed in the future.

Review articles and Drug Reviews

These types of articles should be organised in sections and subsections, as deemed appropriate by the authors

Case Reports and Case Snippets

These types of articles should be organised in the general following sections: Introduction/ Background, Case Report, Discussion. Subsections should be used as deemed appropriate by the authors

Acknowledgements

This section should name everyone who has contributed to the work but does not qualify as an author. People mentioned in this section must be informed and only upon consent should their names be included along with their contributions. Financial support (with grant number, if applicable) should also be stated here.

References

References citation in the text should be numbered sequentially along the text, within square brackets. The use of a reference management tool (such as Endnote or Reference Manager) is recommended. References must be formatted in Vancouver style.

Only published or accepted for publication material can be referenced. Personal communications can be included in the text but not in the references list.

Tables

Tables should be smaller than a page, without picture elements or text boxes. Tables should have a concise but descriptive title and should be numbered in Arabic numerals. Table footnotes should explain any abbreviations or symbols that should be indicated by superscript lower-case letters on the body table.

Figures

Figures should have a concise but descriptive title and should be numbered in Arabic numerals. If the article is accepted for publication, the authors may be asked to submit higher resolution figures. Copyright pictures shall not be published unless the authors submit a written consent from the copyright holder to allow publishing.

Figures should be tested and printed on a personal printer prior to submission. The printed image, resized to the intended dimensions, is almost a replication of how the picture will look online. It shall be clearly perceived, non-pixelated nor grainy. Only flattened versions of layered images are allowed. Each figure can only have a 2-point white space border, thus cropping is strongly advised. For text within figures, Arial fonts between 8 to 11 points should be used and must be readable. When symbols are used, the font information should be embedded.

Photographs should be submitted as *.eps at high-resolution (300 dpi or more), *.tif or *.pdf. Graphics should be submitted in *.eps or *.pdf format, to allow proper reproduction. MS Office graphics are also acceptable, if submitted in their original, editable formats.

Lines, rules and strokes should be between 0.5-1.5 points for reproducibility purposes.

Nomenclature

All units should be in International System (SI). Drugs should be designated by their International Non-Proprietary Name (INN).

3.3. Supporting Information

Code of Experimental Practice and Ethics

The minimal ethics requirements are those recommended by the Code of Ethics of the World Medical Association (Declaration of Helsinki). Authors should provide information regarding ethics on patient informed consent, data privacy as well as competing interests. If the authors have submitted a related manuscript elsewhere, they should disclose this information prior to submission.

3.4. Submission Checklist

Please ensure you have addressed the following issues prior submission:

- Details for competing interests.
- Details for financial disclosure.
- Details for authors contribution.
- Participants informed consent statement.
- Authorisation for use of figures included in the manuscript, not produced by the authors and subject to copyright.
- Authorship, affiliations and email addresses are correct.
- Cover letter addressed to the Editor-in-Chief.
- Identification of potential reviewers and their email addresses (to be introduced at the online submission platform).
- Manuscript, figure and tables comply with the author guidelines, including the correct format, SI units and standard nomenclature.
- Separated files for Title page (*.doc+*.pdf) and Manuscript body (*.doc+*.pdf)—4 in total.
- Manuscript body does not contain the names or affiliations of the authors, or other directly identifying information, and contain the title and the abstract.

If you have any questions, please contact the editorial office at ijcnmh@arc-publishing.org

4. Overview of the Editorial Process

The International Journal of Clinical Neurosciences and Mental Health aims to provide an efficient and constructive view of the manuscripts submitted to achieve a high quality level of publications. The editorial board is constituted by expert leaders in several areas of medicine particularly in Clinical Neuroscience and Mental Health.

Once submitted, the manuscript is assigned to an editor which evaluates and decides whether the manuscript is accepted for peer-review. At this initial phase, the editor evaluates if the manuscript fulfils the scope of the journal according to the content and minimum quality standards. For peer-review, one or two additional expert field editors will comment on the manuscript and decide on whether it is accepted for publishing with minor corrections or not accepted for publishing. The editor may ask authors to resubmit after revision (minor or major). Decision is based on technical and scientific merits of the work. Reviewers can be asked to be disclosed or stay anonymous. Authors can exclude specific editors or reviewers from the process, upon submission, a rational should be provided.

Upon evaluation, an email is sent to the corresponding author with the decision. If accepted, the manuscript enters the production process. It takes approximately 2-4 weeks for the manuscript to be published.

4.1. Appeal Process

The editors will respond to appeals from authors which manuscripts were rejected. Their interests should be sent to the Editor.

Two directions can be followed:

- If the Editor does not accept the appeal, further right to appeal is denied.
- If the Editor accepts the appeal, a further review will be asked. After the new review, the editor can reject or accept the appeal. If rejected, nothing else can be done, if accepted the author is able to resubmit the manuscript.

The reasons for not accepting a manuscript for consideration can be:

- The manuscript does not follow the scope of the journal.
- The manuscript has potential interest but there are methodological concerns after peer-review or editorial examination.